Cyprus University of Technology

Enviromental Bioprocessing Laboratory

LISBOA

Valorisation of Alkaloid Containing Wastewater: Bioconversion of Lupanine into Added-Value Products by Newly Isolated Microorganisms

Stella Parmaki
Ioannis Vyrides
Marlen I. Vasquez
Ioanna Hadjiadamou
Catarina B. M. Barbeitos
Frederico C. Ferreira
Carlos A. M. Afonso
Chryssoula Drouza
Michalis Koutinas

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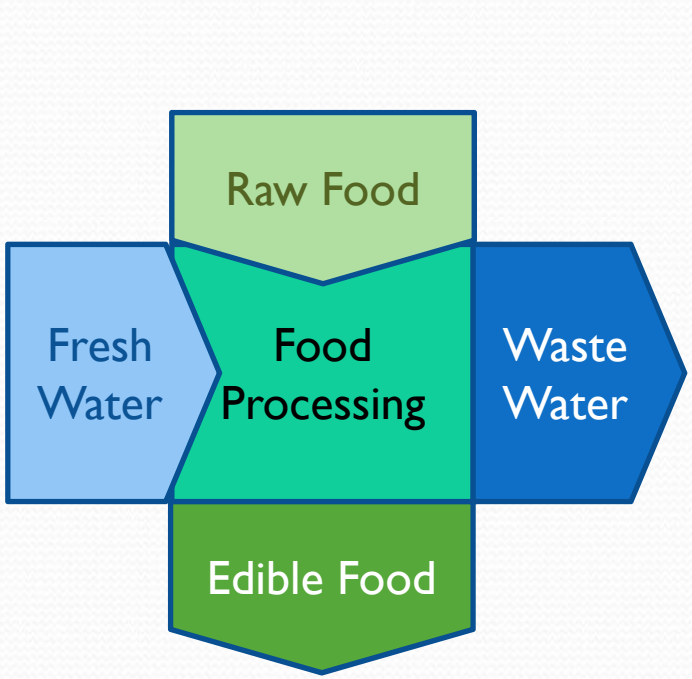
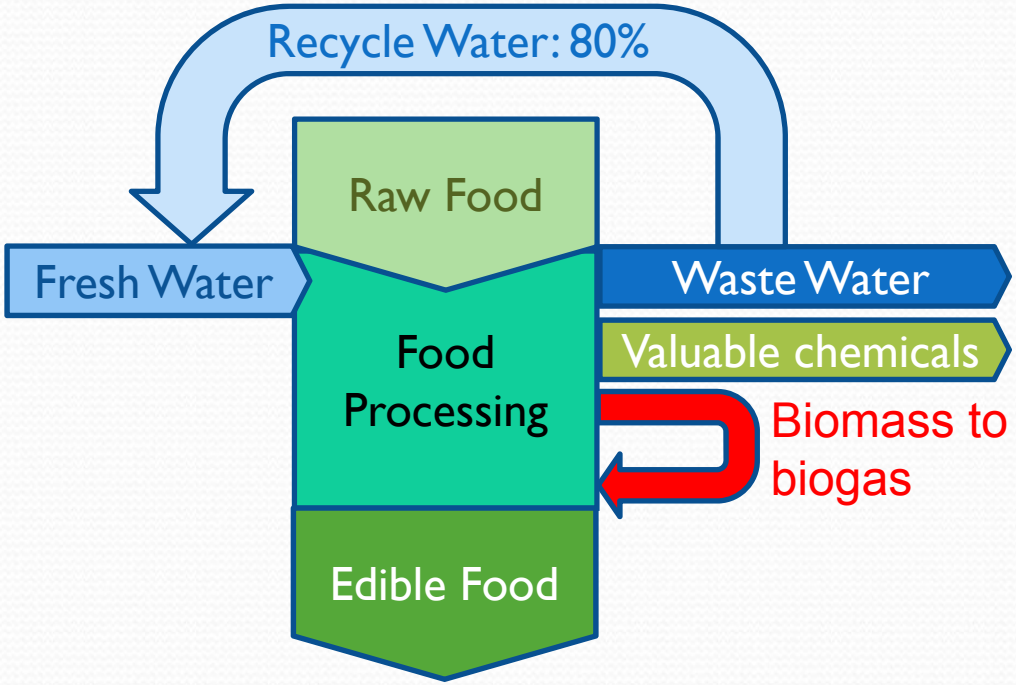
The Concept of Biorg4WasteWaterVal+

Bioorganic Novel Approaches for Food Processing Waste Water Treatment and Valorisation: Lupanine Case Study



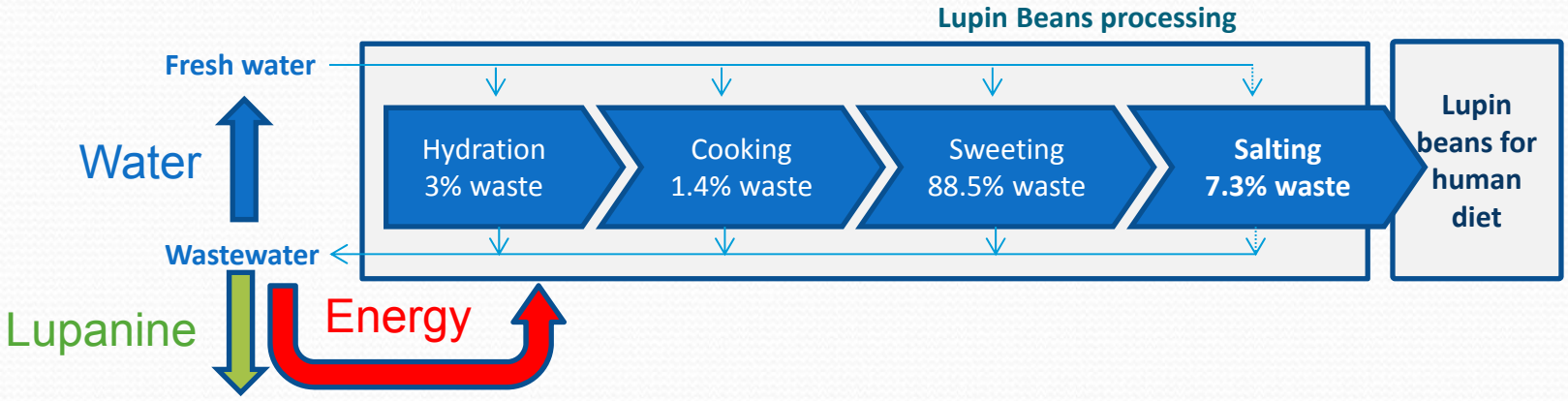
Linear Water Economy

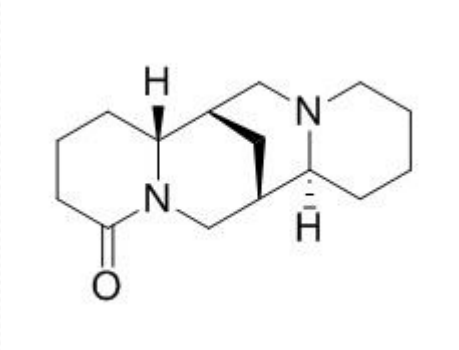


Circular Water Economy

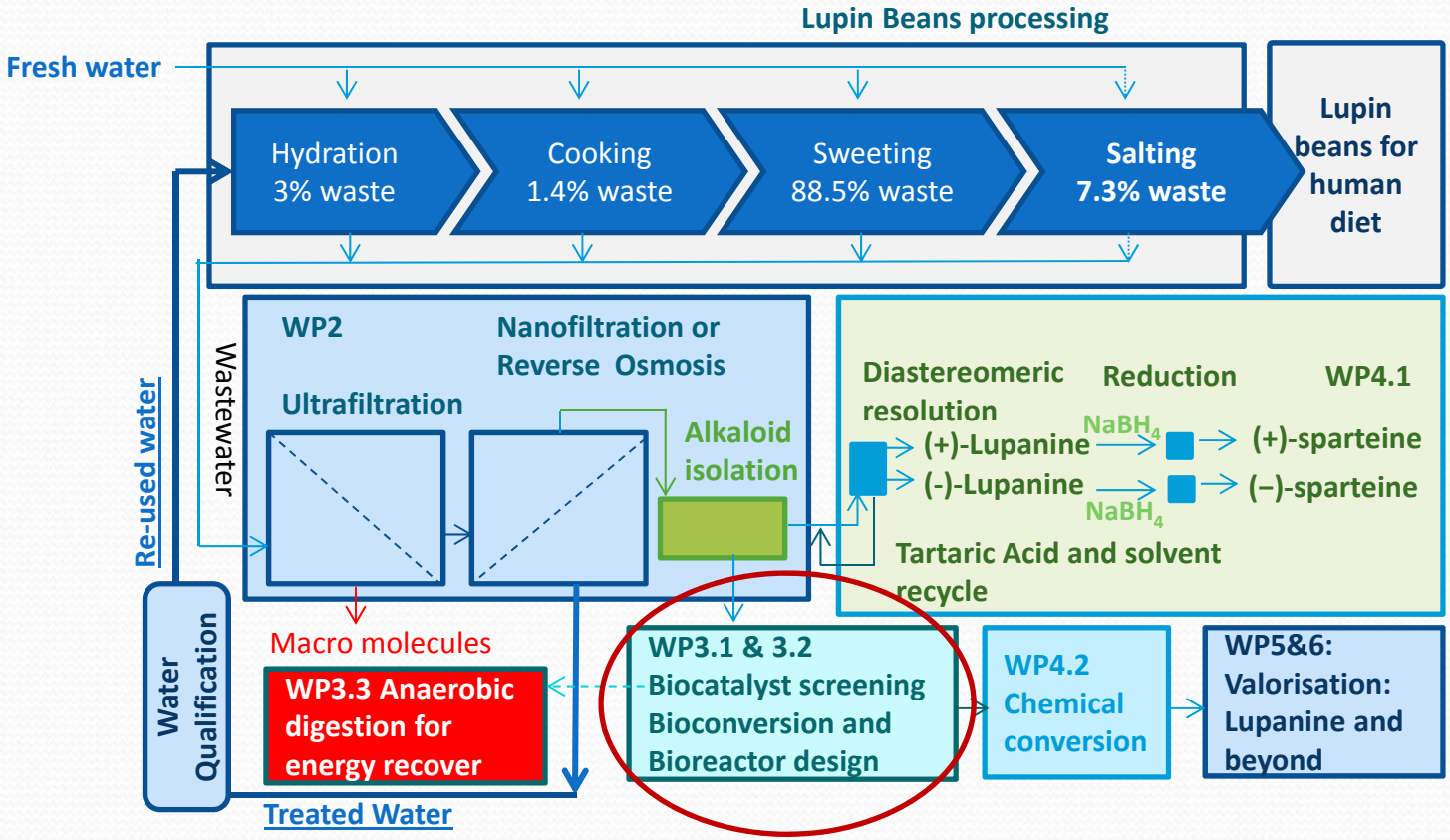
Water JPI

The Lupin Beans Case Study





The Lupin Beans Case Study



Motivation

- Quinolizidine nucleus
- Useful functionalities for fine chemicals and pharmaceutical industries
- Synthesis requires too many steps and the overall yield is low

- Use of a natural molecule as target for biotransformation
- Produce new and known alkaloids with high added-value to overcome laborious total synthesis

Chemical transformation lupanine:

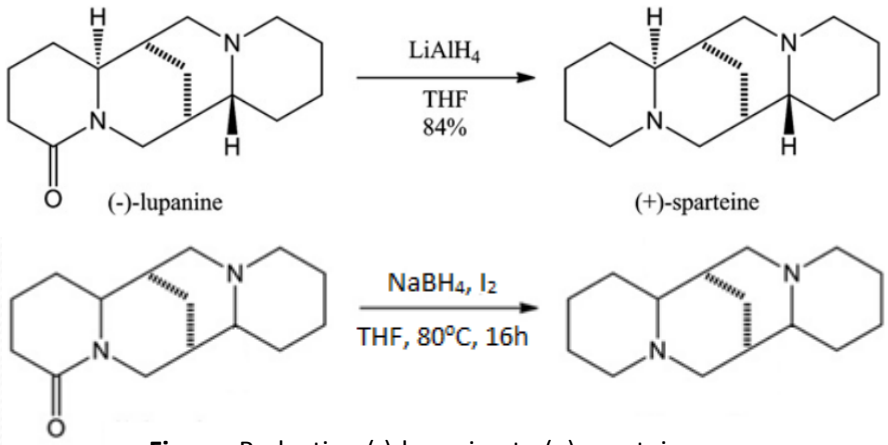


Figure: Reduction (-)-lupanine to (+)-sparteine.

Strains capable of using lupanine:

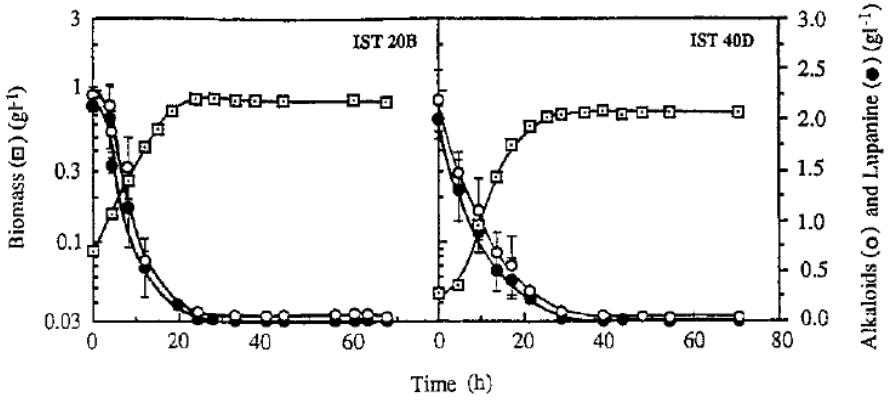


Figure: Decrease of the concentrations of lupanine (●) and total alkaloids (○) during growth (□) of strains IST 20B and IST 40D at 27°C in LUP2 medium.

Lupanine removal (stationary phase): 99%

Strains Metabolising Alkaloids

Nicotine: demethylation pathway in fungi

pyridine pathway in Gram-positive bacteria

pyrolidine pathway in Gram-negative bacteria

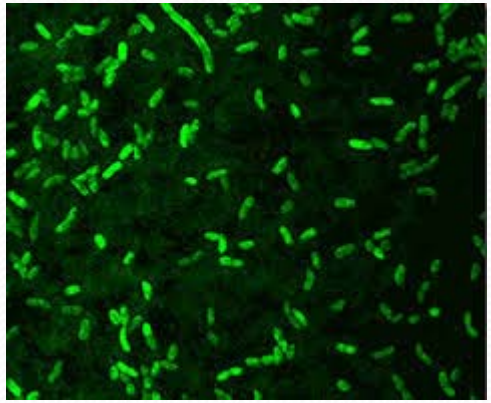
variant of pyridine and pyrolidine pathway in Gram-negative bacteria

Caffeine: *Pseudomonas* sp. CES (9 metabolic enzymes involved)

Lupanine: *Pseudomonas* sp. (lupanine 17-hydroxylase)

Toxicological Aspects of Lupanine - Aquatic

Vibrio fischeri

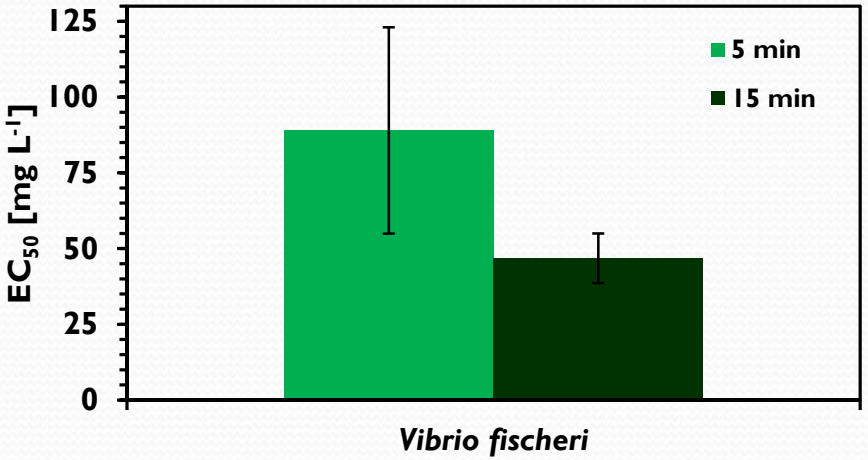


Marine bacteria

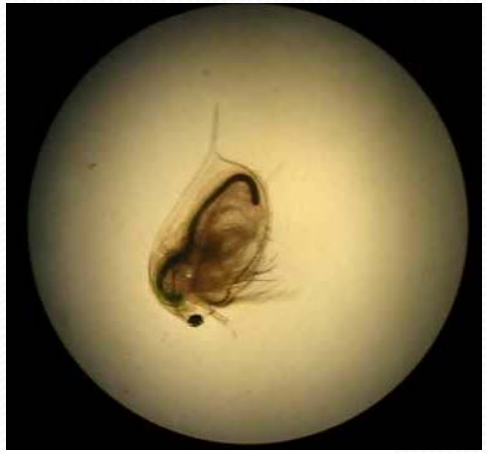
Luminescence inhibition

Highly toxic

T= 21°C, Light



Daphnia magna

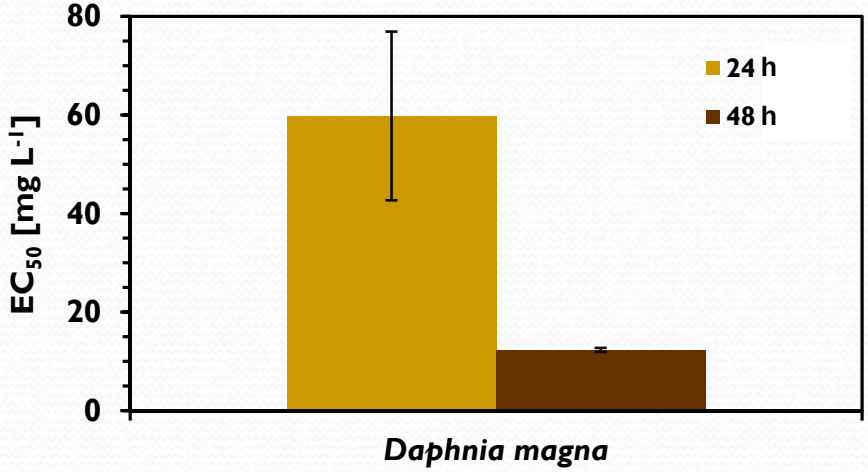


Planktonic crustacean

Freshwater organism

Immobilisation test

Highly toxic



Toxicological Aspects of Lupanine - Plants

T= 21°C, Dark

Sinapis alba

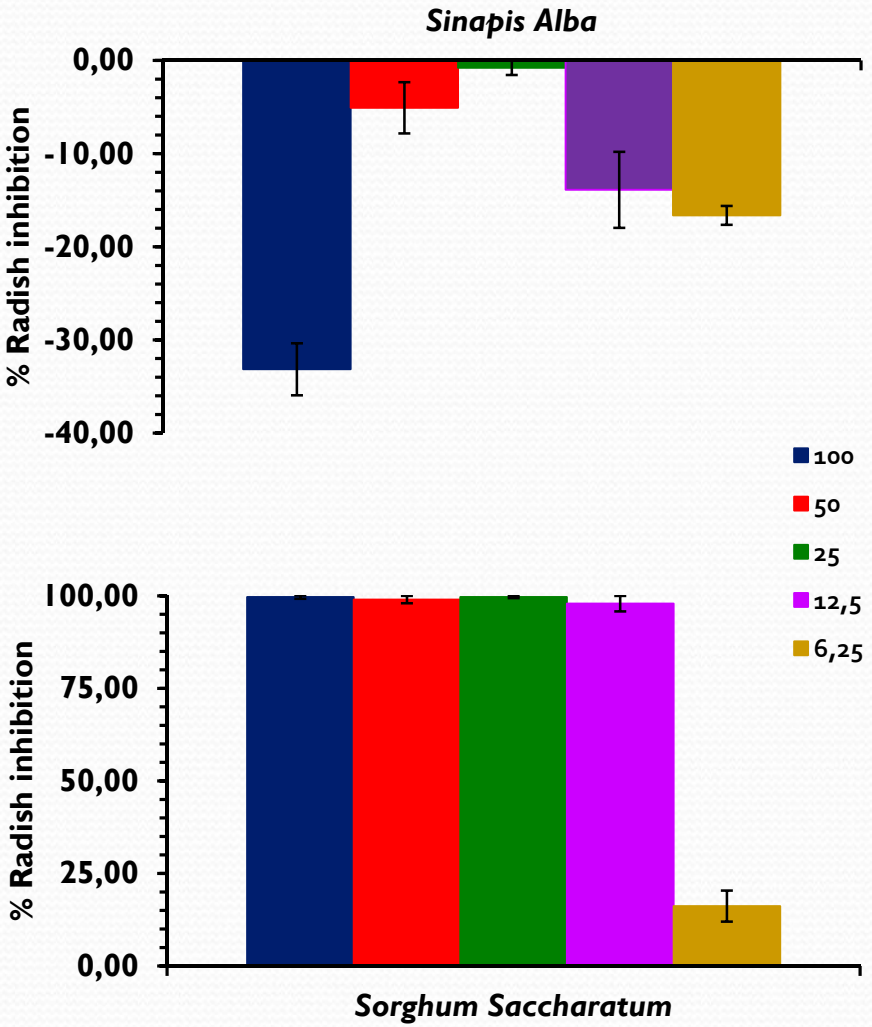
Dicotyledonous seeds

Radicle growth

Positive effect

Non-toxic

Lupinus albus is dicotyledonous



Sorghum sacchratum

Monocotyledonous seeds

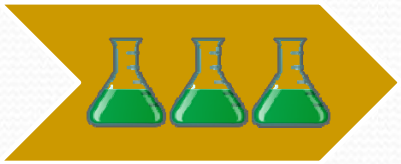
Radicle growth

Negative effect

Highly toxic

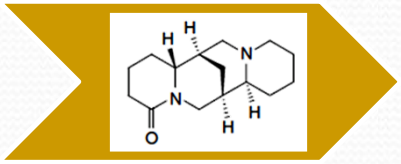
Isolation of Lupanine Metabolising Strains

Environmental Samples



Granular Sludge
Anaerobic Sludge
Aerobic Sludge
Lupinus Wastewater

Carbon Source



Lupanine

1 g L^{-1} lupanine, 30 °C, pH 7

Aerobic

Granular Sl. Digested Sl. Aerobic Sl. Lupinus WW

Anaerobic

Granular Sl. Granual Sl. Digested Sl. Digested WW

8 Microbial Isolates

1.5 g L^{-1} lupanine, 30 °C, pH 7

Aerobic

Anaerobic

16S rRNA Sequencing (Macrogen – The Netherlands)

TGAATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCAGCTTGCTGGGTGGATT
AGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTTCGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATA
GGACCTCGGGATGCATGTTCCGGGGTGGAAAGGTTTTCCGGTGCAGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGGGGT
AACGGCCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCT
TCGGGTTGTAAACCTCTTTCAGTACCGACGAAGCGCAAGTGACGGTAGGTACAGAAGAAGCACCGGCCAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGTCT
GTGAAAACCCGCAGCTCAACTGCGGGCTTGCAGGCGATACGGGCAGACTTGAGTACTGCAGGGGAGACTGGAATTCCTGGT
GTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACTGACGCTGAGGAGC
GAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCGCTAGGTGTGGGTTTCCTTCCA
CGGGATCCGTGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATACACCGGAC
CGCCCCAGAGATGGGGTTTCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCACGTAATGGTGGGGACTCGCAGGAGACTGCCGGGGTCAA
CTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCCGGTACAGA
GGGCTGCGATACCGCGAGGTGGAGCGAATCCCTTAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAA
GTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCAT
GAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCCTCGTGGGAA

Aerobic

Rhodococcus rhodochrous
Rhodococcus sp.
Rhodococcus rubber
Pseudomonas putida

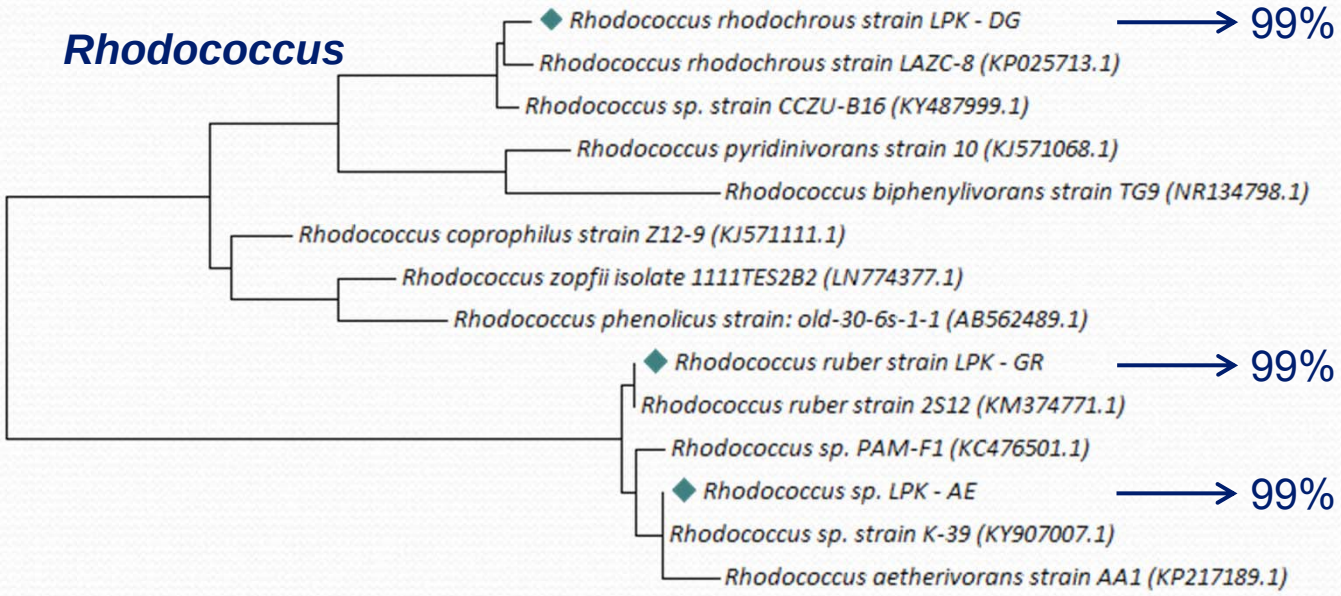
Anaerobic

Rhodobacter sp.
Ochrobactrum tritici
Pseudomonas citronellolis
Pseudomonas sp.

Water JPI

Phylogenetic Trees of Isolates

Rhodococcus



Alkaloids (Berberine, Ergopeptines, Nicotine)

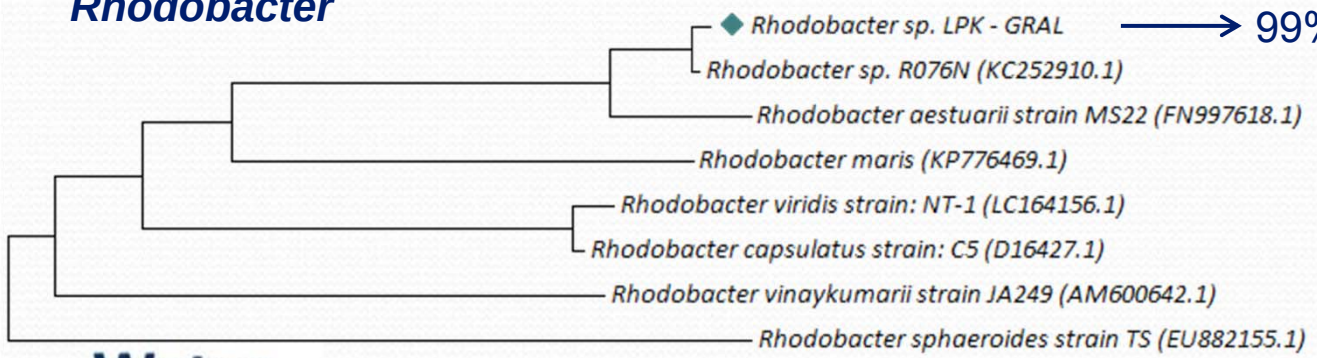
Nitroaromatics (e.g. nitrophenol)

Aliphatics (e.g. *n*-hexadecane)

Aromatics (e.g. *o*-xylene)

Polycyclic Aromatic Hydr. (PAH)

Rhodobacter



Nitroaromatics

Aliphatics

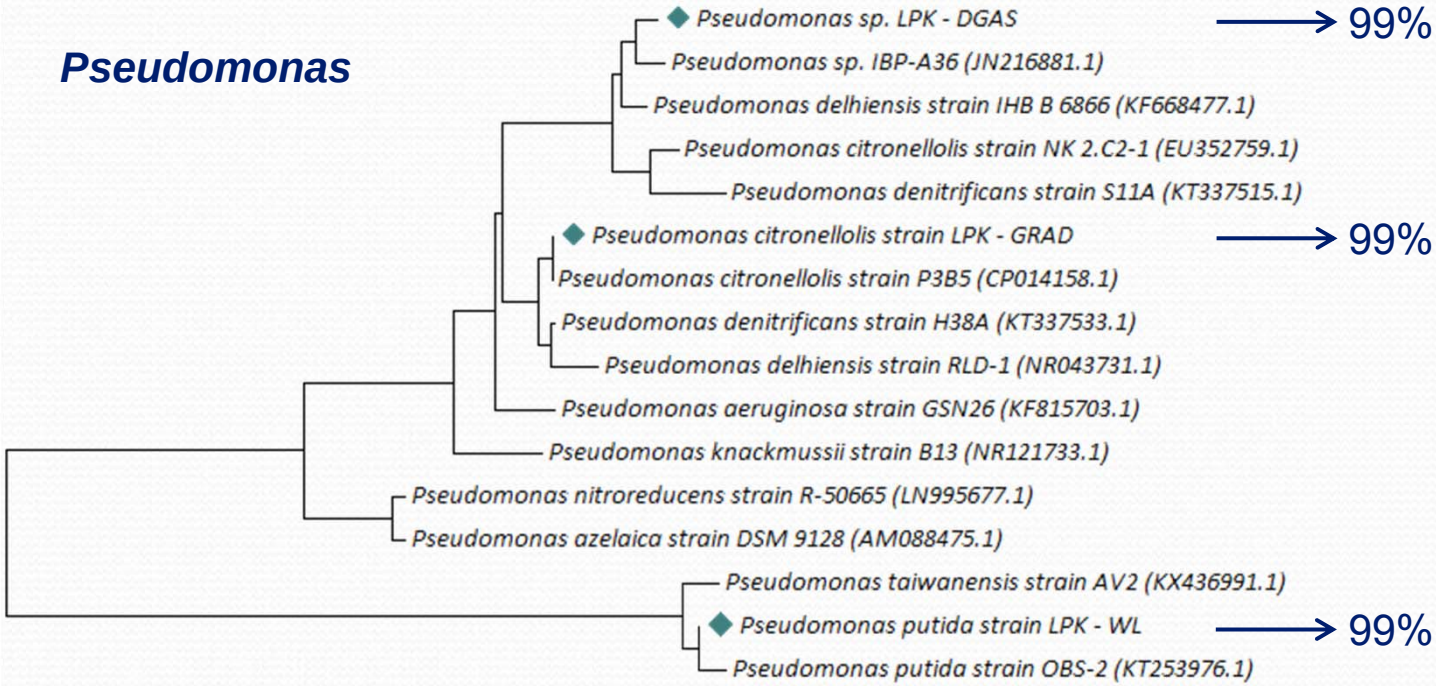
Aromatics

Polycyclic Aromatic Hydr. (PAH)

Water JPI

Phylogenetic Trees of Isolates

Pseudomonas



99%

99%

99%

Versatile degrader

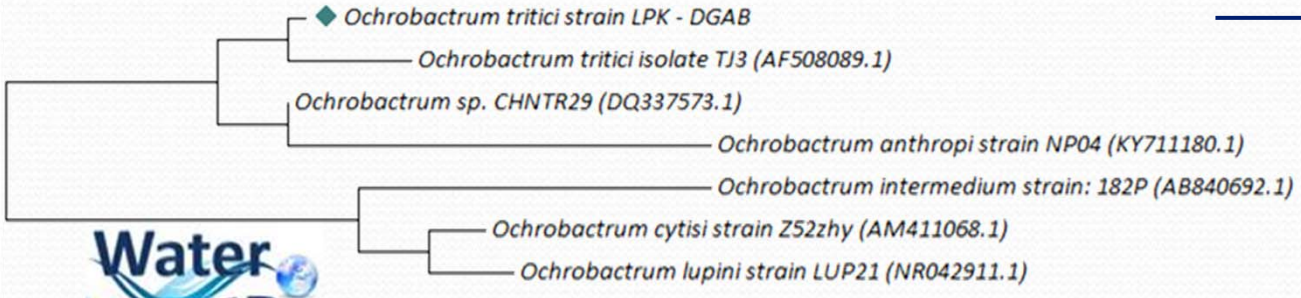
Alkaloids (Caffeine, Nicotine)

Aromatics (e.g. benzene)

Herbicides (e.g. atrazine)

Isoprenoids (e.g. citronellol)

Ochrobactrum



99%

Alkaloids (Nicotine)

Aliphatics

Aromatics

Polycyclic Aromatic Hydr. (PAH)

Water JPI

Lupanine Biodegradation – Aerobic Strains

Conditions: 31 °C, pH 7, minimal medium (M9)

Stationary phase
P. putida LPK411: 30 h
Other 3 strains: 36 h

% Removal

R. rhodochrous LPK211: **80%**

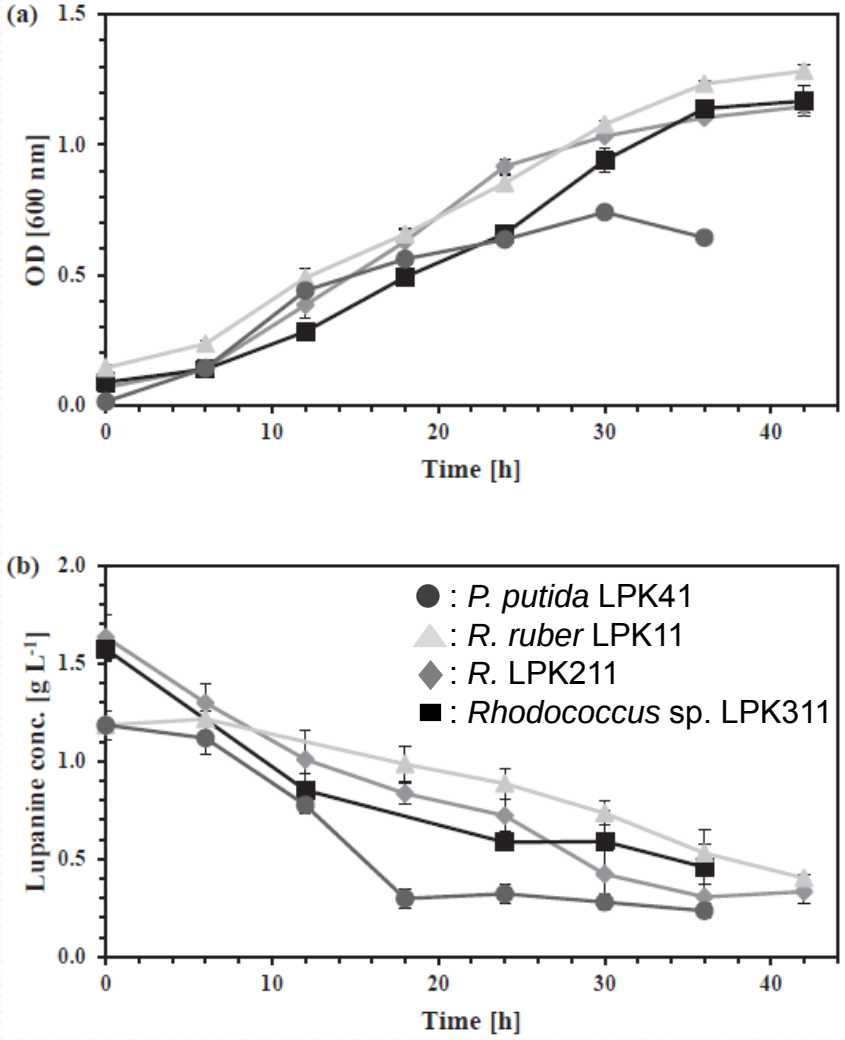
R. sp. LPK311: **70%**

R. ruber LPK111: **69%**

Other studies

1 g L^{-1} removed (99%) in 10 h from wastewater (Santana et al. 2002)

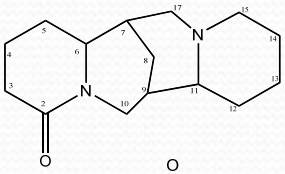
3 g L^{-1} removed (99%) in 30 h from wastewater (Santana et al. 1996)



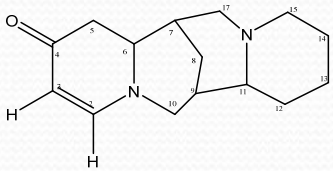
Santana F.M.C. et al., (2002) *J. Agric. Food Chem.* 50:2318-2323.
Santana F.M.C. et al., (1996) *J. Ind. Microbiol.* 17:110-115.

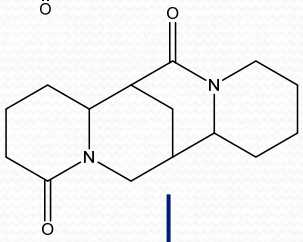
Final Metabolic Products – Aerobic Strains

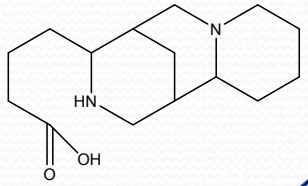
Lupanine



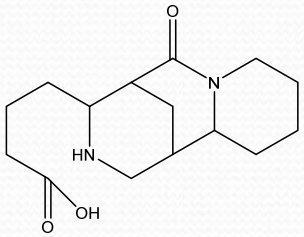
Multiflorine



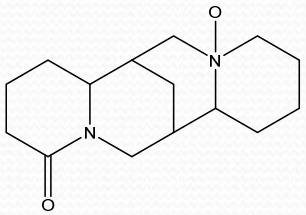




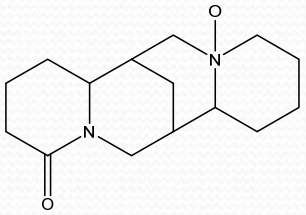
New generation sparteine analogues via alkylation on the amide bond



***P. putida* LPK411**



***R. ruber* LPK111**



***R.* sp. LPK311**

Complete Bioconversion

***R. rhodochrous* LPK211**

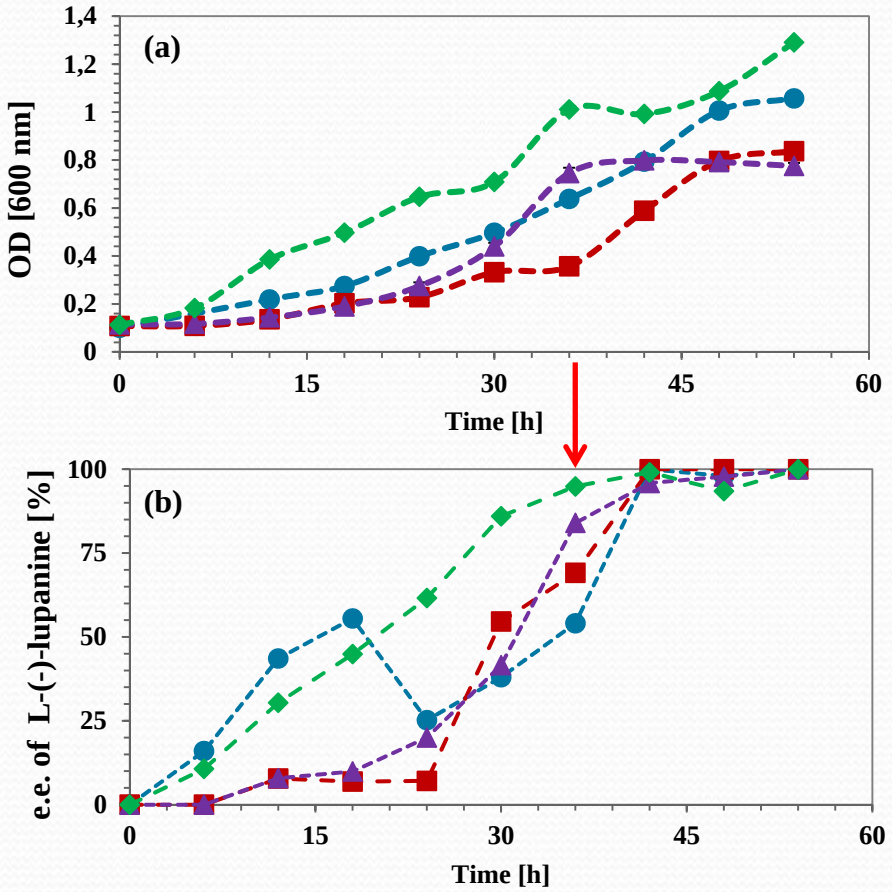
Resolution of Racemic Lupanine

Racemic mixture: D-(+)-lupanine, L-(-)-lupanine

Conditions: 31 °C, pH 7, minimal medium (M9)

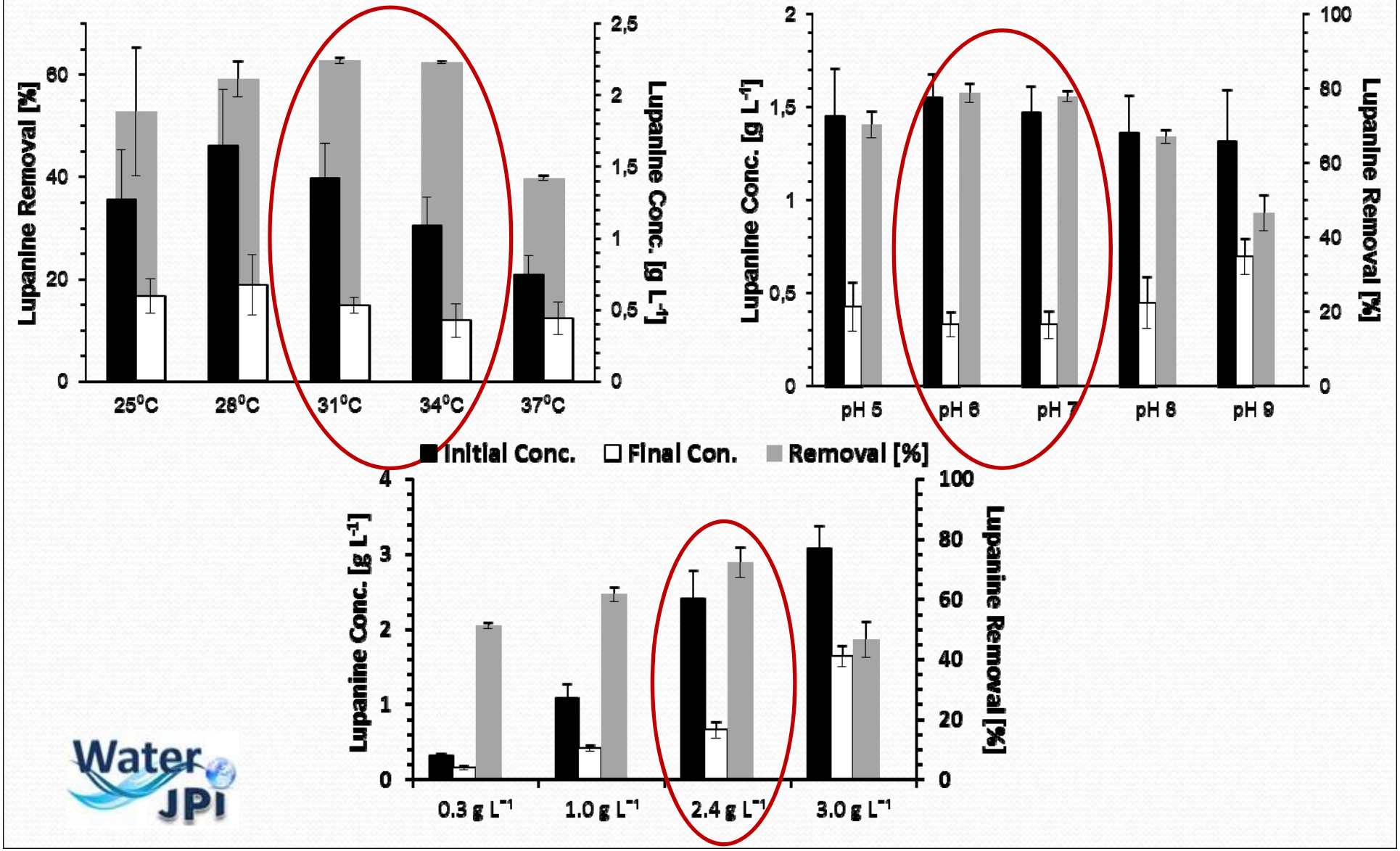
All strains e.e. 95-100% at **42 h**
P. putida LPK411: e.e. 95% at **36 h, 53% lupanine**

L-(-)-lupanine: synthesis of **L-(-)-sparteine**



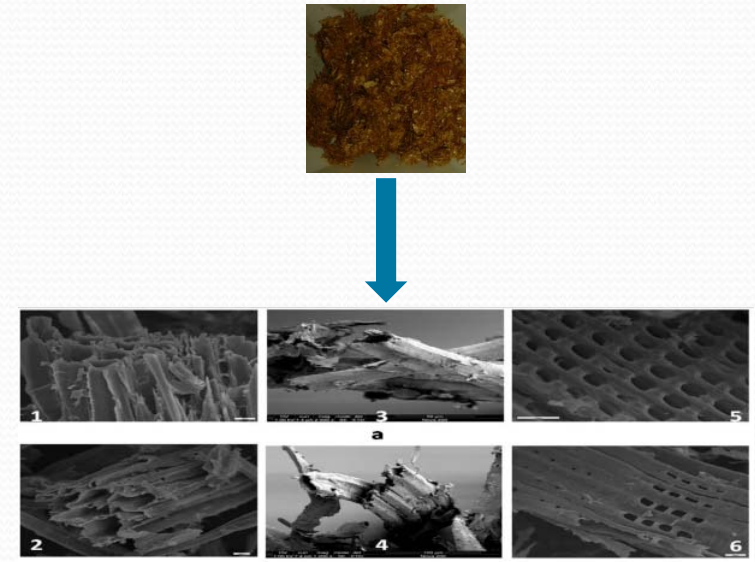
◆ : *P. putida* LPK41
■ : *R. ruber* LPK11
● : *R.* LPK211
▲ : *Rhodococcus* sp. LPK311

Optimisation of *P. putida* Growth on Lupanine



Future Opportunities

- Microbial kinetics and metabolic products from each enantiomer
- Immobilization on microbial supports
- Bioreactor studies



Water JPI

Conclusions

- Lupanine is **highly toxic** for aquatic organisms
- **Non-toxic** for dicotyledonous
- Bioconversion of lupanine under **aerobic** conditions
- Useful metabolic **end-products**
- *P. putida* performs **resolution** of racemic lupanine

Thank You!


Water
2014
Works


Life


Research
Promotion
Foundation


Water
JPI